Research Notes

Effect of plant extracts derived from thyme on male broiler performance

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ABSTRACT The effect of dietary thyme-oil extract (TOE) supplementation on immune functions of broilers were assessed by feeding graded levels (50, 100, 200, or 400 ppm) of TOE to male broiler chicks during a 42-d feeding trial compared with negative- or positive-control diets. Dietary control treatments included a negative-control diet with no feed-additive supplementation and 2 positive-control groups supplemented with either virginiamycin or zinc bacitracin. In total, 300 1-day-old Ross × Ross male broilers were randomly assigned to 6 dietary treatments that consisted of 5 replicates of 10 birds each. On d 21 and 42, 2 birds from each replicate were killed by cervical cutting to measure the relative weights of spleen and bursa of Fabricius. At 25 d of age, chicks were injected with 0.5 mL of 10% SRBC suspension. Broilers fed with 200 ppm of TOE had heavier weights of bursa of Fabricius than those fed other dietary treatments at d 42 of age. Furthermore, dietary inclusion of 100 ppm of TOE resulted in higher ($P < 0.05$) total immunoglobulin response in primary antibody titer against sheep erythrocytes compared with other dietary treatments. On the other hand, diet modifications had no significant effect on blood leukocyte subpopulations and heterophil-to-lymphocyte ratio. These results suggest that dietary supplementation with TOE, especially at the level of 100 ppm, can improve immunological responses of broiler chicks.

Key words: broiler chick, thyme-oil extract, immune response, lymphoid organ

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INTRODUCTION

The use of antibiotics in poultry feed improves performance and morbidity in broiler chickens. However, consumer pressure related to the potential development of antibiotic-resistant bacteria in human populations has resulted in the development of antibiotic-alternate feed additives in animal rations that may also improve broiler performance (Buchanan et al., 2008). Essential oils, organic acids, and phytogenic compounds such as thyme oil are important antibiotic alternatives that have been demonstrated to enhance production of gastric secretions and reduce pathogenic bacteria (Barnes et al., 1994; Wenk, 2000; Taylor, 2001).

Essential oils should be regarded as one of several available feed additives that have been shown to have antibacterial activity against undesirable pathogenic bacteria, such as *Salmonella* spp. and others (Elgayyar et al., 2001). Essential oils consist of several active compounds, with some of them comprising more than 60 individual components that can inhibit the growth of certain microorganisms (Russo et al., 1998). The chemical composition of essential oils is variable. For example, the concentrations of the main components of thyme essential oil (thymol and carvacrol) can range from 3 to 60% of the total essential oil (Lawrence and Reynolds, 1984; Aeschbach et al., 1994). Major components can constitute up to 85% of the essential oil, whereas other components are present only as a trace (Senatore, 1996; Bauer et al., 2001); nevertheless, they are also very important. The primary components are the major active ingredients, whereas the secondary components act synergistically to increase the total effectiveness (Tzakou et al., 2001).

Thyme (*Thymus vulgaris* L.) is a popular medicinal plant mostly grown in Mediterranean regions and is among the herbaceous plants that have received increased attention due to its antioxidant and antibacterial properties. The herb has also been reported to have antibacterial activities against a wide range of pathogenic microbial organisms (Varel, 2002). In addition, these phenolic compounds exhibit considerable antimicrobial and antifungicidal activities (Basilico and Basilico, 1999). Al-Kassie (2009) reported that chicks fed with 200 ppm of essential oil (**EO**) derived from thyme and cinnamon had significantly higher feed intake, BW gain, and feed conversion ratio, followed by chicks fed with 100 ppm of EO derived from thyme and cinnamon, compared with control

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Table 1. Ingredients and nutrient composition of basal diets during starter and grower periods.

Item	Starter (1–21 d)	Grower (21–42 d)
Ingredient		
Corn, yellow	54.68	62.25
Soybean meal	38.19	31.30
Sunflower oil	3.00	2.14
Dicalcium phosphate	1.85	1.36
Limestone	1.23	1.30
Common salt	0.40	0.30
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-Methionine	0.15	0.05
Nutrient composition		
ME (kcal/kg)	3,000	3,030
CP (%)	21.57	18.94
Methionine (%)	0.49	0.36
Lysine (%)	1.18	1.01
Calcium (%)	0.94	0.85
Nonphytate P (%)	0.43	0.34

[1]Supplied per kilogram of diet: vitamin A (retinyl acetate), 15,000 IU; vitamin D3, 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 mg; vitamin K, 5 mg; thiamin, 3 mg; riboflavin, 10 mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 70 mg; folic acid, 2 mg; biotin, 0.4 mg; pantothenic acid, 20 mg.

[2]Supplied per kilogram of diet: manganese, 80 mg; zinc 75 mg; iron, 70 mg; copper, 10 mg; iodine, 2 mg; selenium, 0.3 mg.

group that showed the lowest performance. Furthermore, Cross et al. (2007) reported that dietary thyme had a different effect on weight gain and body mass when used as an herb or oil. However, there is only limited evidence about whether its inclusion as a solid herb material would have a growth-promoting effect in live birds. To our knowledge, there has been limited research conducted with thyme-oil extract (TOE) to investigate its effects on immunological responses of poultry. Therefore, the objective of this study was to determine the effects of dietary TOE fortification on immune functions of male broiler chicks.

MATERIALS AND METHODS

Birds and Diets

In total, 300 1-day-old male broiler chicks (Ross × Ross 308) were weighed and randomly distributed into 5 replicates (10 chicks in each) each of 6 treatments, so that all dietary treatments had a similar mean weight and weight distribution. Randomization of experimental pens was performed according to a completely randomized design. A corn-soybean meal basal diet (Table 1) was prepared in mash form and formulated to meet or exceed the nutrient requirements of broiler chickens recommended by the National Research Council (NRC, 1994). Dietary control treatments included the negative control group with no feed additive and a positive control supplemented with zinc bacitracin antibiotic. Oil extract derived from thyme at the levels of 50, 100, 200, or 400 ppm was compared with the control treatments. All birds were kept under similar conditions of management throughout the experimental period. Artificial lighting was used to provide chicks with 24 h of light. Initial brooding temperature was 33°C in the first week of age and reduced gradually by 3°C per wk until the birds were 4 wk old. Feed and water were provided for *ad libitum* consumption. Daily feed consumption and daily BW gains were recorded weekly, and mortality was recorded as it occurred to correct feed-conversion data.

Chemical Analysis

Prior to formulating the diets, feed ingredients were analyzed for crude protein, ether extract, crude fiber, and ash contents according to standard AOAC (1995) procedures. After that, the experimental diets were formulated by means of these determined actual values.

Antibody Response to SRBC

At d 25 of age, 2 randomly selected chicks from each pen replicate were peritoneally injected with 0.5 mL of a 10% saline suspension of SRBC. Blood samples were collected from each bird at d 7 after SRBC challenge. Immediately after blood sampling, the same 2 birds were given a secondary challenge (0.5 mL, 10% SRBC), and blood samples were collected again 7 d later to quantify anti-SRBC antibody titers. The total, mercaptoethanol-sensitive (**MES**, presumably IgM) and mercaptoethanol-resistant (**MER**, presumably IgY) anti-SRBC antibody titers were measured using a microhemagglutination technique as previously described by Qureshi and Havanstein (1994). The antibody data were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible agglutination.

Heterophil-to-Lymphocyte Ratio

At d 42, blood samples were obtained via wing vein of 2 birds per pen and collected into the tubes containing EDTA as an anticoagulant. Two drops of blood were placed on the slide, and blood smear was prepared using Giemsa staining method (Lucas and Jamroz, 1961). All slides were coded, and heterophils and lymphocytes were counted to a total number of 100 cells per slide.

Lymphoid Organ Weights

At the end of the experiment (42 d of age), 2 birds from each replicate were selected randomly, weighed, and exsanguinated after anesthesia. Bursa Fabricius and spleen were collected. Spleen and bursa Fabricius weights were recorded immediately after collection.

Statistical Analysis

Statistical analysis was performed using the GLM procedures of SAS statistical software (SAS Institute, 2002). A completely randomized design was considered

Table 2. Effect of dietary thyme-oil extract (TOE) concentration on lymphoid organ weights (% of live weight) of broiler chicks at 21 and 42 d of age.

Treatment	Supplemental level (mg/kg of diet)	Bursa of Fabricius		Spleen	
		21 d	42 d	21 d	42 d
Control	0	0.246[a,b]	0.160[d]	0.128	0.169
Zinc bacitracin	200	0.231[b]	0.188[b]	0.096	0.158
Virginiamycin	200	0.244[b]	0.176[c]	0.093	0.187
TOE	50	0.261[a]	0.145[e]	0.101	0.147
	100	0.188[c]	0.159[d]	0.108	0.167
	200	0.248[a,b]	0.200[a]	0.092	0.191
	400	0.235[b]	0.147[e]	0.099	0.135
P-value		0.0001	0.0001	0.3825	0.2852
SEM		0.0054	0.0041	0.0114	0.0175
Contrast					
Negative control vs. TOE		0.0457	0.4129	0.0528	0.6643
Positive controls vs TOE		0.0325	0.0001	0.5186	0.4263

[a-e]Means with no common superscript within each column are significantly ($P < 0.05$) different.

for ANOVA, and pen was the experimental unit for all measurements. All treatment means were compared by Duncan's multiple-range tests (Duncan, 1955), and differences were separated at the statistical $P < 0.05$ level. The single-degree-of-freedom contrasts were made among the treatment means to compare the negative control versus TOE and 2 positive controls versus TOE treatments.

RESULTS AND DISCUSSION

To better understand the mechanisms by which TOE could beneficially affect immune functions, we evaluated the influences of TOE on lymphoid organ weight, antibody responses to SRBC injections, and peripheral blood distribution of leukocyte subpopulations. The effect of dietary supplementation with TOE or antibiotic on lymphoid organ development in 3- and 6-wk-old broilers is presented in Table 2. In 21-d-old birds, dietary supplementation with 50 and 200 ppm of TOE increased ($P < 0.05$) the relative weight of bursa of Fabricius, but dietary inclusion of either 400 ppm of TOE or positive control (zinc bacitracin) had no effect when compared with the negative control group (without supplementation). In 42-d-old broilers, dietary supplementation with 200 ppm of TOE increased ($P < 0.05$) the relative weight of bursa of Fabricius compared with the positive and negative controls. On the other hand, in groups in which feed was supplemented by 100 ppm thyme oil, the weight of the bursa of Fabricius was diminished in comparison to other TOE levels. In other words, 100 ppm thyme oil had better effects on birds' bursa of Fabricius weight. In both 21 and 42 d-aged chicks, dietary treatments had no marked ($P > 0.05$) effect on the relative weight of spleen.

Of all the immunological parameters assessed, the morphometric measures that examined bursa weight and the bursa-to-BW ratio gave the most consistent and reliable indication of stress. This is a commonly assessed measure in studying the immune system of the bird and is easily accessible at slaughter (Pope, 1991). Toghyani et al. (2010) suggest that supplementing broilers' diet with 5 g/kg of thyme can indicate favorable influences of antibiotic growth promoter on performance without any detrimental effects on immune responses and blood parameters. In addition, the positive effect of EO was also observed during in vivo trial in newly weaned piglets, where a combination of cinnamon, thyme, and oregano extracts was used to investigate their effect on growth performance, gut morphology, microbiota, and immune response (Namkung et al., 2004).

The anti-SRBC titers for total immunoglobulins, IgM, and IgY were measured by hemagglutination assay (Table 3). Except for total antibody titer in primary antibody response to SRBC, no significant difference was found between dietary treatments. Dietary supplementation with 100 ppm of TOE resulted in higher ($P < 0.05$) concentrations of total immunoglobulins in primary antibody titers against SRBC in comparison with other dietary treatments. Contrast comparisons showed that TOE versus antibiotic supplementation had a significant effect ($P < 0.05$) on total anti-SRBC antibody titers in secondary response. Incremental levels of TOE reduced total anti-SRBC immunoglobulins. It seems that TOE might have resulted in correlated changes in T-cell subpopulations, therefore affecting antibody production. With increasing dietary TOE supplementation, we observed that a high concentration of TOE (200 and 400 ppm) failed to additionally increase the antibody response to SRBC vaccination in comparison to the lower levels. This effect may be explained by the results in human dendrite cells showing that excess antioxidant can downregulate the immune response (Verhasselt et al., 1999). Since TOE likely acts as an antioxidant (Placha et al., 2014), it is possible that high doses could prevent initial increase of reactive oxidant species that are necessary to stimulate lymphocytes of broiler chickens.

Moreover, dietary supplementation with TOE compared with antibiotic treatment increased ($P < 0.05$)

Table 3. Effect of dietary thyme-oil extract (TOE) concentration on antibody response to SRBC (log_2).

Treatment	Supplemental level (mg/kg of diet)	Primary titer			Secondary titer		
		IgT[1]	IgM	IgY	IgT	IgM	IgY
Control	0	4.8[b,c]	2.4	2.4	5.2	1.6	3.6
Zinc bacitracin	200	5.8[a,b]	3.6	2.2	4.0	1.8	2.2
Virginiamycin	200	5.6[a,b]	4.0	1.6	5.6	2.4	3.2
TOE	50	4.4[c]	3.0	1.4	6.0	0.8	5.2
	100	6.2[a]	3.8	2.4	5.8	1.4	4.4
	200	5.6[a,b]	2.6	3.0	5.8	2.0	3.8
	400	5.2[a,b,c]	2.8	2.4	5.6	2.4	3.2
P-value		0.0171	0.1070	0.1690	0.1471	0.7180	0.4911
SEM		0.3464	0.4488	0.4208	0.5126	0.7289	0.9942
Contrast							
Negative control vs. TOE		0.1666	0.2058	0.8332	0.3042	0.9515	0.6240
Positive controls vs. TOE		0.2532	0.0638	0.2818	0.0323	0.4819	0.1033

[a–c]Means with no common superscript within each column are significantly ($P < 0.05$) different.

[1]Immunoglobulin Total.

Table 4. Effect of dietary thyme-oil extract (TOE) concentration on blood leukocyte subpopulation.

Treatment	Supplemental level (mg/kg of diet)	Heterophil (%)	Lymphocyte (%)	H:L[1]
Control	0	24.7	69.25	0.356
Zinc bacitracin	200	25.0	70.0	0.356
Virginiamycin	200	25.7	70.25	0.364
TOE	50	24.7	70.5	0.349
	100	24.5	70.75	0.345
	200	25.0	70.5	0.354
	400	24.0	71.25	0.336
P-value		0.6727	0.6996	0.5483
SEM		0.6551	0.8600	0.0098
Contrast				
Negative control vs. TOE		0.8392	0.1154	0.3729
Positive controls vs. TOE		0.1571	0.6539	0.1100

[1]Heterophil to lymphocyte ratio.

SRBC antibody titers during secondary response. The changes pattern in peripheral blood leukocyte subpopulations is observed in Table 4. As noted, dietary treatments had no significant effect on proportion of heterophils and lymphocytes, as well as heterophil-to-lymphocyte ratio. A low ratio indicates low levels of stress, but it remained unaffected in our study.

In conclusion, TOE promotes humoral immune responses in broiler chicks. Moreover, dietary supplementation of TOE at the levels of 100 ppm in replacement of antibiotics can improve overall immune functions in birds. However, further research is needed to quantify and specify the effects of these herbal components on cellular and humoral immune functions of broiler chicks at the molecular level.

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